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Sullivan & Worcester LLP 1666 K Street, N.W. Washington, DC 20006			EXAMINER BHAT, NARAYAN KAMESHWAR	
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

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<b>Office Action Summary</b>	<b>Application No.</b> 10/563,195	<b>Applicant(s)</b> TODD ET AL.	
	<b>Examiner</b> NARAYAN K. BHAT	<b>Art Unit</b> 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 16 June 2010.
- 2a) ☒ This action is **FINAL**.                      2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 1-38 is/are pending in the application.
- 4a) Of the above claim(s) 38 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-37 is/are rejected.
- 7) ☒ Claim(s) 15 is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- |   |   |
|---|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892)                    | 4) <input type="checkbox"/> Interview Summary (PTO-413)           |
| 2) <input type="checkbox"/> Notice of Draftperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____                                      |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)         | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____   | 6) <input type="checkbox"/> Other: _____                          |

### **FINAL REJECTION**

1. This action is in response to the paper filed on June 16, 2010. The previous rejections made in the office action mailed on December 18, 2009 are maintained. Applicant's arguments filed on June 16, 2010 have been fully considered and are not found persuasive as discussed below following the claim rejections. Accordingly, ***this action is made final.***

### ***Claim Status***

2. Claims 1-38 are pending in this application. Claim 21 has been amended. The claim amendments have been reviewed and entered.
3. Claim 38 is withdrawn from further consideration as being drawn to a nonelected invention in the reply filed on May 18, 2007 and made final in the office action dated June 20, 2008.
4. This application contains claim 38 drawn to an invention nonelected with traverse in the reply filed on December 19, 2008. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action ((37 CFR 1.144) See MPEP § 821.01).
5. Claims 1-37 are under prosecution.

***Claim Objections***

6. The previous objection to claim 21 has been withdrawn in view of placing a missing comma between “Apolipoprotein B mRNA editing enzyme” and “Activation – Induced cytidine Deaminase”.

Claim 15 is objected to because of the following informalities: Claim 15 is identified as “currently amended” without any amendments. Applicant is reminded that all claims being currently amended be submitted with markings to indicate the changes that have been made relative to the immediate prior version of the claims. The text of any added subject matter must be shown by underlining the added text. The text of any deleted matter must be shown by strike-through except that double brackets placed before and after the deleted characters may be used to show deletion of five or fewer consecutive characters. The text of any deleted subject matter must be shown by being placed within double brackets if strike-through cannot be easily perceived (See MPEP, 714 [R6] for further guidance). Appropriate correction is required.

***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

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the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 1-4, 14-31, 33 and 35-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107, cited in the IDS filed 4/3/2006).

***The previous rejections are maintained.***

Regarding claim 1, Gitan et al teaches a method for detecting the presence or level of alkylated cytosine in a sample of genomic double stranded DNA from an individual comprising following steps.

Regarding step 'a', Gitan et al teaches obtaining a sample of the genomic double stranded DNA from the individual (pg. 162, Methods section, pg. 162, column 2, and paragraph 3).

Regarding step 'b', Gitan et al teaches converting at least one region of the double stranded DNA to single stranded DNA by treating with mild heat and alkali/sodium bisulfite using Intergen kit (Gitan et al, pg. 162, column 2, paragraph 4, Intergen kit, pg. 6, Modification protocol, pg. 7. step II).

Regarding step 'c', Gitan et al teaches that the single stranded DNA comprises both methylated (i.e., alkylated) and unmethylated cytosine and bisulfite treatment

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converts unmethylated cytosine to uracil, whereas methylated cytosine remain unchanged in the single stranded DNA (Fig. 1, pg. 159, column 1, Results section, paragraph 1), which encompasses differentially modifying alkylated cytosine and cytosine present in the single stranded DNA. Methylated cytosine of Gitan et al is the alkylated cytosine as defined in instant claim 35.

Regarding step 'd', Gitan et al teaches determining the level of bisulfite modification of the target region by analyzing converted (i.e., unmethylated) and unconverted (i.e., methylated alleles) alleles on an oligonucleotide array (Fig. 1, bottom panel, pg. 159, results section paragraph 1). Gitan et al do not teach an enzyme differentially modifying alkylated cytosine and cytosine present in the single stranded DNA.

Regarding claims 2 and 3, Gitan et al teaches that the single stranded DNA is reacted with the bisulfite under conditions such that the bisulfite reacts substantially only with cytosine (Fig. 1, pg. 159, column 1, Results section, paragraph 1). Gitan et al do not teach an enzyme reacting with cytosine present in the single stranded DNA.

Regarding claim 4, Gitan et al teaches that the conversion of the region of the double stranded DNA to the single stranded DNA comprises separating the two strands of the double stranded DNA by treating with mild heat and alkali/sodium bisulfite using Intergen kit (Gitan et al, pg. 162, column 2, paragraph 4, Intergen kit, pg. 6, Modification protocol, pg. 7. step II).

Regarding claim 14, Gitan et al teaches that the determination of the level of bisulfite modification of the single stranded DNA with single stranded comprises

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analyzing for methylated and unmethylated cytosine sequence variations arising from the modification of the target region of the single stranded DNA by the bisulfite (Fig. 4, see the legend).

Regarding claim 15, Gitan et al teaches the PCR amplification of the selected target region using PCR to obtain an amplified product and analyzing the amplified product by sequence variation (Fig. 1, See the legend for details).

Regarding claim 16, Gitan et al teaches that the analysis of the amplified product comprises subjecting the amplified product to a technique involving the use of probes that bind to specific nucleic acid sequences (Fig. 1, bottom panel).

Regarding claim 17, Gitan et al teaches that the analysis of the amplified product comprises subjecting the amplified product to a polymerase chain reaction technique with nested primers (pg. 162, column 1, and paragraph 1).

Regarding claims 18-21, Gitan et al teaches that bisulfite deaminates cytosine present in the target regions of the single stranded DNA (Fig. 1) but do not teach an enzyme deaminating cytosine.

Regarding claim 22, Gitan et al teaches detecting the presence of methylated cytosine in an ER alpha gene (Fig. 4).

Regarding claim 23, Gitan et al teaches detecting the presence of methylated cytosine in a promoter region, i.e., "5' untranslated region of a gene" (pg. 162, column 1, paragraph 1, lines 1-2).

Regarding claim 24, Gitan et al teaches that the level of methylated cytosine comprises hypermethylation (Figs. 4 and 5 and pg. 160, column 1, paragraph 2).

Regarding claim 25, Gitan et al teaches the levels of methylated cytosine in the ER alpha gene is lower in MCF-7 cells than in the MDA-MB-231 cells (Fig. 4, pg. 160, column 1, paragraph 2), which encompasses the levels of methylated cytosine comprise hypomethylation.

Regarding claim 26, Gitan et al teaches that the gene is ER alpha (Abstract).

Regarding claims 27-31, Gitan et al teaches detection of altered level of alkylated cytosine in the target region of the single stranded DNA is a marker for cancer (Abstract and pg. 161, column 1, paragraph 2).

Regarding claim 33, Gitan et al teaches that the variable methylation of the ER alpha CpG island in normal fibroblast strain HFF results in silencing of the ER alpha gene expression (Fig. 5, pg. 160, column 1, last paragraph and pg. 161, column 1, first paragraph), which encompasses the level of methylated cytosine indicating the absence of an altered gene imprinting state.

Regarding claim 35 and 36, Gitan et al teaches that the alkylated cytosine is 5-methyl cytosine (pg. 158, column 2, lines 1-2 and pg. 163, column 2, reference of Frommer et al).

Regarding claim 37, Gitan et al teaches that the double stranded DNA is a genomic DNA (pg. 162, Method section, and paragraph 1).

As described above, Gitan et al do not teach reacting single stranded DNA with enzyme that differentially modulate alkylated cytosine and cytosine present in the single stranded DNA.

However, an enzyme differentially modifying alkylated cytosine and cytosine was known in the art at the time of the claimed invention was made as taught by Bransteitter et al.

Bransteitter et al teaches a method, wherein an Activation Induced cytidine Deaminase (AID) enzyme that modulates the activity of single strand DNA comprising cytosine and methylated cytosine differentially by 10-fold (Fig. 4b, Abstract and pg. 4106, column 1, paragraph 4).

Regarding claim 18, Bransteitter et al teaches that AID enzyme deaminates cytosine in the target region of the single stranded DNA (Fig. 1, single strand DNA labeled as ssDNA).

Regarding claim 19, Bransteitter et al teaches that AID enzyme and RNaseA (i.e., combination of enzymes) are employed to differentially modify 5-methyl cytosine (i.e., alkylated cytosine) and cytosine in the target region (Fig. 4B, pg. 4106, column 1, and last paragraph).

Regarding claims 20 and 21, Bransteitter et al teaches that the AID enzyme is a deaminase enzyme having deaminase activity (Fig. 1).

Bransteitter et al also teaches that AID enzyme differentially modifies single stranded DNA containing 5-methyl cytosine than cytosine (Fig. 4b, pg. 4106, column 1, paragraph 4) and further teaches that the deamination is very rapid and completes in minutes (Fig. 4A, pg. 4106, column 1, last paragraph).

Both Gitan et al and Bransteitter et al are interested in understanding the importance of methylation pattern in biological processes and therefore method steps

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are combinable. As described above, Gitan et al teaches that the bisulfite treatment take long hours to differentially modulate the single stranded DNA comprising unmethylated and methylated cytosine, where as Bransteitter et al teaches that an enzyme treatment takes minutes to differentially modulate single stranded DNA comprising unmethylated and methylated cytosine. One having skill in the art would like to use an enzyme for detecting methylation status in a target genome, because it requires less time and does not require additional steps of purifying target as taught by Bransteitter et al.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to modify the bisulfite deamination step of Gitan et al with the AID enzyme deamination step of Bransteitter et al with a reasonable expectation of success.

An artisan would have been motivated to modify the bisulfite deamination step of Gitan et al with the expected benefit of using AID enzyme for detecting methylation status of the target genome in minutes as taught by Bransteitter et al (Fig. 4A and 4B, pg. 4106, column 1, paragraph 4), thus expediting the detection of methylation pattern of cancer causing genes using the methylation specific oligonucleotide microarray of Gitan et al.

10. Claims 5-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164) in view of Bransteitter et al (PNAS,

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2003, 100, 4102-4107) as applied to claims 1 and 4 as above and further in view of Kuhn et al (J. Am. Chem. Soc., 2002, 124, 1097-1103).

***The previous rejections are maintained.***

The teachings of Gitan et al and Bransteitter et al regarding claims 1 and 4 are described above in section 9.

Regarding claims 5-13, Bransteitter et al teaches generating bubble comprising single stranded region in the double stranded region using partially complementary double stranded DNA (Table 1, legend). Gitan et al and Bransteitter et al do not teach using strand displacing probes to separate the two strands of the double stranded DNA. However, partially separating the two strands of the double stranded DNA with strand displacing probes were known in the art at the time of the claimed invention was made as taught by Kuhn et al.

Regarding claim 4, Kuhn et al teaches a method for opening of the double stranded DNA with PNA openers to partially separate the two strands of the double stranded DNA (Fig. 5A, right panel, pg. 1101, column 1, and paragraph 2).

Regarding claim 5, Kuhn et al teaches a method wherein PNA openers (i.e., strand displacing probes) are used to partially separate the two strands of the double stranded DNA (Fig. 5A, right panel, pg. 1101, column 1, paragraph 2).

Regarding claim 6, Kuhn et al teaches that the strand displacing probes are PNA containing probes (Fig. 5A, right panel, pg. 1101, column 1, and paragraph 2).

Regarding claim 7, Kuhn et al teaches a method wherein the double stranded DNA is opened with PNA openers and further teaches that PNA openers forms triplexes

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and exposes the displaced DNA strand for binding with other DNA and PNA beacons (Fig. 5A and B, right panel, pg. 1101, column 1, paragraph 2), thus teaching inhibiting annealing of the two strands of the double stranded DNA together once they have been separated to facilitate access to the target region by the enzyme. With regard to the recitation of “to facilitate access to the target region by the enzyme”, the phrase is the property of the DNA being at least partially separated, which Kuhn et al teaches.

Regarding claim 8, Kuhn et al teaches hybridizing at least one PNA beacon probe with a strand of the double stranded DNA following separation of the two strands to form a triplex structure, thereby inhibit the annealing of the two strands together (Fig. 5A and B, right panel, pg. 1101, column 1, and paragraph 2).

Regarding claim 9, Kuhn et al teaches that the PNA probe is antisense probe (Fig. 5B, right panel, Fig. 7A, See the legend, pg. 1102, column 2, paragraph 1).

Regarding claim 10, Kuhn et al teaches at least two PNA opener probes are hybridized with the strand of the double stranded DNA, one of the probes hybridizing with a region of the strand downstream of the target region (Fig. 5A, right panel, see the PNA opener at the right side of the displaced strand) and other probe hybridizing with a region of the strand upstream of the target region (Fig. 5A, right panel, see the PNA opener at the left side of the displaced strand).

Regarding claim 11, Kuhn et al teaches wherein the probe hybridizes with upstream and downstream regions of the strand which flank the target region such that a loop which incorporates the target region is formed in the strand (Fig. 5A, right panel, see the loop formed by two PNA openers).

Regarding claim 12, Kuhn et al teaches that the probe hybridizes with the strand of the double stranded DNA either side of the target region (Fig. 5A, right panel) and further teaches that the probe has a middle region of non-complementary sequence that does not hybridize with the target region such that a loop incorporating the target region is formed in the strand (Fig. 5A, right panel).

Regarding claim 13, Kuhn et al teaches that the middle region of the probe incorporates inverted repeats that hybridize together following hybridization of the probe with the strand of the double stranded DNA (Fig. 5A, right panel).

Kuhn et al also teaches that PNA beacons are insensitive to the presence of salt and DNA-binding/processing proteins and have a great potential as robust tools for recognition of specific sequence within double strand DNA without denaturation and deproteinization of duplex DNA (Abstract).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to modify the step of converting double stranded DNA into single stranded DNA of Gitan et al with the PNA openers of Kuhn et al with a reasonable expectation of success.

An artisan would have been motivated to modify the step of converting double stranded DNA into single stranded DNA of Gitan et al with the expected benefit of using PNA beacons, which are insensitive to the presence of salt and DNA-binding/processing proteins and have a great potential as robust tools for recognition of specific sequence within double strand DNA without denaturation and deproteinization of duplex DNA as taught by Kuhn et al (Abstract).

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11. Claim 32 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107) as applied to claim 1 as above and further in view of Opdecamp et al (Nucleic Acids Research, 1992, 20, 171-178).

***The previous rejections are maintained.***

The teachings of Gitan et al and Bransteitter et al regarding claim 1 are described above in section 9.

Regarding claim 32, Gitan et al teaches the presence of methylated cytosine in human foreskin fibroblast (Fig. 5). Gitan et al and Bransteitter et al do not teach levels of methylated cytosine to indicate the presence or absence of fetal cells. However, levels of methylated cytosine to indicate the presence or absence of fetal cells were known in the art at the time the invention was made as taught by Opdecamp et al.

Opdecamp et al teaches a method for detecting the presence or level of alkylated cytosine in a sample of genomic double stranded DNA from an individual, the method further comprising the detection of an altered level of alkylated cytosine in fetal liver than in adult liver (Fig. 5, Compare the pattern of fetal and adult liver), which encompasses the level of the alkylated cytosine to indicate the presence of fetal DNA.

Opdecamp et al also teaches that higher gene expression in fetal than in adult cells is due to methylation pattern of unmethylated site in the non-coding portion of the gene in fetal and adult cells (Abstract).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to apply the method of detecting methylation pattern in

cancer cells of Gitan et al to detect the presence of fetal cells as suggested by Opdecamp et al with a reasonable expectation of success.

An artisan would have been motivated to detect fetal cells by the method of Gitan et al with the expected benefit of detecting genes that are expressed in fetal cells are also expressed in cancer cells as taught by Opdecamp et al (Abstract).

12. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107) as applied to claim 1 as above and further in view of Paulson et al (J. Virol., 1999, 73, 9959-9968).

Claim 34 is dependent from claim 1. The teachings of Gitan et al and Bransteitter et al regarding claim 1 are described above in section 9.

Regarding claim 34, Gitan et al teaches that the methylated cytosine in multiple CpG island loci changes in breast cancer (Abstract). Gitan et al and Bransteitter et al do not teach that the presence of alkylated cytosine for indicating the presence or absence of a pathogen or a microorganism.

However, the presence of the alkylated cytosine indicating the presence or absence of a pathogen or a microorganism was known in the art at the time the invention was made as taught by Paulson et al.

Paulson et al teaches that the EBV is the etiologic agent of infectious mononucleosis, i.e., a "pathogen" (pg. 9959, column 1, paragraph 1) and further teaches that the presence of EBV is characterized by the presence of methylation at its

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promoter sites (Fig. 3, pg. 9964, column 2 paragraph 2). Paulson et al also teaches that the EBV usurps the host cell directed methylation system to regulate pathogen gene expression and thereby establish a chronic infection (Abstract).

It would have been prima facie obvious to one having the ordinary skill in the art at the time the invention was made to apply the method of detecting methylation pattern in cancer cells of Gitan et al for detecting the presence of pathogen as suggested by Paulson et al with a reasonable expectation of success.

An artisan would have been motivated to detect pathogen by the method of Gitan et al with the expected benefit of detecting expression of genes that are induced by pathogen EBV as taught by Paulson et al (Abstract, pg. 9959, column 1, and paragraph 1) are also induced or repressed in cancer cells.

### ***Response to Remarks from the Applicants***

#### ***Rejections under 35 U.S.C. § 103(a)***

13. Applicant's arguments filed June 16, 2010 with respect to claims 1-4, 14-31, 33 and 35-37 as being unpatentable over Gitan et al in view of Bransteitter et al have been fully considered (Remarks, pgs. 8-10) but they are not persuasive for the following reasons.

Applicant's arguments are directed to: (i) there is no disclosure in Gitan et al of use of an enzyme which differentially modifies methylated cytosine and unmethylated cytosine in single stranded DNA (Remarks, pg. 8, paragraph 3), (ii) the deficiency of Gitan is not cured by Bransteitter (Remarks, pg. 8, paragraph 4) and (iii) Bransteitter is

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non-analogous art and a person of ordinary skill in the art would not have considered this reference in order to solve the problem of providing a new method for detecting the presence or level of alkylated cytosines in a sample of genomic or mitochondrial DNA (Remarks, pg. 8, paragraph 4).

With respect to argument (i), the Examiner acknowledges that there is no disclosure in Gitan et al of use of an enzyme which differentially modifies methylated cytosine and unmethylated cytosine in single stranded DNA. However, the Applicant is reminded that the claimed method steps are rejected under 35 USC 103 (a) using a combination of references of Gitan et al in view of Bransteitter et al. Bransteitter et al teaches AID enzyme which differentially modifies methylated cytosine and unmethylated cytosine in single stranded DNA within 5 minutes, thus providing teachings , suggestions and motivations for one having skill in the art to use an enzyme for differentially modulating methylated cytosine and unmethylated cytosine in single stranded DNA, because it requires less time than the bisulfite treatment in the method of Gitan et al. For these reasons, argument (i) is not persuasive.

With respect to argument (ii), it is noted that the Applicant has acknowledged that Gitan et al teaches the method steps 'a' to 'd' except for the enzyme, which differentially modifies methylated cytosine and unmethylated cytosine in single stranded DNA and Bransteitter is broadly directed to an enzyme treatment to differentially modulate DNA comprising methylated and unmethylated cytosine (Remarks, pg. 8 paragraphs 3 and 4). Also, as described above in section 9, Both Gitan et al and Bransteitter et al are interested in the field of methylation in biological processes and therefore method steps

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are combinable. Gitan et al teaches that the bisulfite treatment takes long hours to differentially modulate single stranded DNA comprising unmethylated and methylated cytosine, whereas Bransteitter et al teaches that an enzyme treatment takes minutes to differentially modulate single stranded DNA comprising unmethylated and methylated cytosine. One having skill in the art would like to use an enzyme for detecting methylation status in a target genome, because it requires less time and does not require additional steps of purifying the target as taught by Bransteitter et al. Since proposed method steps of Gitan and Bransteitter are easy to combine, arguments that the deficiency of Gitan is not cured by Bransteitter are not persuasive.

Applicants further argue that Bransteitter et al do not teach a method for determining the presence or levels of alkylated cytosine in DNA. Applicants further assert that method of Gitan and Bransteitter are different field of endeavor and are nonanalogous art (Remarks, pg. 8, paragraph 4). These arguments are not persuasive because as described above in section 9, step of measuring the level of alkylated cytosine is taught by Gitan et al and the teachings of Bransteitter et al are relied only for an enzyme that differentially modulate single stranded DNA comprising unmethylated and methylated cytosine.

With respect to argument (iii), that Bransteitter et al is nonanalogous art, it has been held that a prior art reference must either be in the field of applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the applicant was concerned, in order to be relied upon as a basis for rejection of the claimed invention (See *In re Oetiker*, 977 F.2d 1443, 24 USPQ2d 1443 (Fed. Cir. 1992)). In this

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case, as described above and in section 9, Gitan et al and Bransteitter et al teaches modifying, detecting the level of alkylated cytosine to gain information on the methylation pattern in biological processes. Thus the references are in the field of methylation and therefore deemed to be an analogous art. It is also maintained that the reference of Bransteitter et al is an analogous art and one having skill in the art would like to apply an enzyme for detecting methylation status in a target genome, because it requires less time and does not require additional steps of purifying target as taught by Bransteitter et al.

Applicant further assert that the abstract of Bransteitter et al is completely silent on the issue of methylation, and, in fact, it concludes only that AID catalyses deamination of dC residues on single stranded DNA in vitro but not on double stranded DNA, RNA-DNA hybrids or RNA (Remarks, pg. 9, paragraph 1). Applicant's assertions are noted. However, instant claim 1 as recited requires reacting a target region of the single stranded DNA from step (b) with at least one enzyme, the enzyme differentially modifying alkylated cytosine and cytosine present in the single stranded DNA (Emphasis added by the Examiner with an underline). Furthermore, Bransteitter explicitly teaches that "our data showing that AID (i.e., Activation Induced Deaminase) catalyzes dC → dU deamination activity most avidly on dsDNA containing a 9-nt "transcription-like" ssDNA bubble" (pg. 4106, column 2, paragraph 2, last sentence). Thus, Applicant's argument that Bransteitter et al not teaching deamination of dC residues on double stranded DNA are not commensurate in scope of the claim. For these reasons, Applicant's alleged assertions are not persuasive.

Applicant further assert that the experiments described on page 4106 and Figure 4b were designed to confirm and extend the previous finding that the sole activity observed for AID was with a free nucleoside (CR) or deoxynucleoside CdR substrate, and that this activity was not reliant upon the pre-treatment of RNAses. The aim of the experiments described in this paragraph was to elucidate the need for RNAses, not the measurement of methylation patterns (Remarks, pg. 9, paragraph 2).

Applicant's assertions are noted. However, as described above and in section 9, the teachings of Bransteitter et al are relied only for an enzyme that differentially modulate single stranded DNA comprising unmethylated and methylated cytosine and Gitan et al teaches the measurement of methylation patterns. Furthermore, Bransteitter explicitly teaches that "our data showing that AID (i.e., Activation Induced Deaminase) catalyzes dC → dU deamination activity most avidly on dsDNA containing a 9-nt "transcription-like" ssDNA bubble" (pg. 4106, column 2, paragraph 2, last sentence). For these reasons, Applicant's alleged assertions are not persuasive.

Based on the alleged assertions, Applicant reiterate that one of ordinary skill in the art would not have considered Bransteitter et al to be "reasonably pertinent" to their particular problem and, therefore, would not have identified this reference (Remarks, pg. 9, paragraph 5). This argument is repetitive and is not persuasive for the same reasons as described above.

Applicant further argues that a reference putatively teaching a means by which an enzyme contributes to somatic hyper mutation is NOT reasonably pertinent to the particular problem with which the present inventors were involved, namely the

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development of novel methods for measuring methylation (Remarks, pg. 9, last paragraph). These arguments are repetitive and are not persuasive for the same reasons as described above.

Applicant further argues that in the present situation, the present invention is in the field (industry) of measuring methylation patterns on DNA, whereas the prior art is in the field (industry) of B cell immunology. Therefore, it is for at least these reasons that Bransteitter is non-analogous art, and can therefore not be combined with the teachings of Gitan et al. to render the present invention as allegedly obvious (Remarks, pg. 10, paragraph 2). These arguments are repetitive and are not persuasive for the same reasons as described above.

Applicant further argues that the Examiner has not established a prima facie case of obviousness. Applicants further assert that the conclusory statements that both references would be combined because they are both "interested in understanding the importance of methylation pattern in biological processes..." and "one having skill in the art would like to use an enzyme." do not suffice as "articulated reasoning." As such, Applicant respectfully requests withdrawal of this rejection. (Remarks, pg. 10, paragraph 3). These arguments are not persuasive because as described above and in section 9, Gitan et al teaches that the bisulfite treatment takes long hours to differentially modulate single stranded DNA comprising unmethylated and methylated cytosine, where as Bransteitter et al teaches that an enzyme treatment takes minutes to differentially modulate single stranded DNA comprising unmethylated and methylated cytosine. One having skill in the art would like to use an enzyme for detecting methylation status in a

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target genome, because it requires less time and does not require additional steps of purifying target as taught by Bransteitter et al. Since proposed method steps of Gitan and Bransteitter are easy to combine, arguments are not persuasive. It is also noted that Applicant has presented similar arguments after the final office action, which have been addressed fully in the advisory action mailed on July 24, 2009.

Applicant's remaining arguments regarding the teachings of Kuhn et al, Opdecamp and Paulson et al are directed towards not curing the deficiency of Gitan et al and Bransteitter et al (Remarks, pgs. 11-12). These arguments are not persuasive because as described above and in section 9, Gitan et al in view of Bransteitter et al teach recited steps 'a' to 'd' and therefore it is maintained that the claimed method steps are obvious over Gitan et al in view of Bransteitter et al and the teachings of Kuhn et al are relied only for separating two strands of double stranded DNA with different means including strand displacing probes. Similarly the teachings of Opdecamp et al are relied only for the presence or level of the alkylated cytosine indicating the presence or absence of fetal DNA and the teachings of Paulson et al are relied only for the presence or level of the alkylated cytosine indicating the presence or absence of a pathogen.

### ***Conclusion***

14. No claims are allowed.

15. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Narayan K. Bhat whose telephone number is (571)-272-5540. The examiner can normally be reached on 8.30 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on (571)-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Customer Service Representative or access to the automated information system, call  
800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Narayan K. Bhat/

Examiner, Art Unit 1634

/Stephen Kapushoc/

Primary Examiner, Art Unit 1634